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IR-spectroscopy and PLS modelling for advanced bio-process monitoring: simultaneous and rapid sensing of various components

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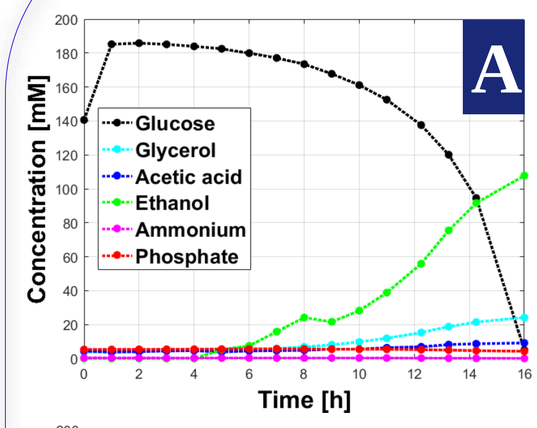
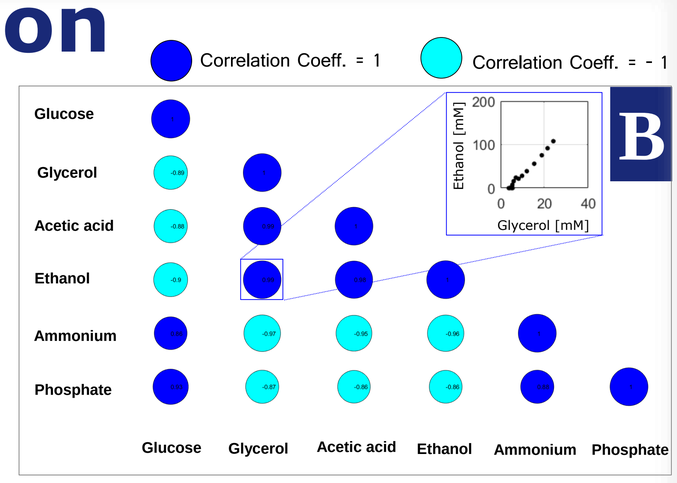
Introduction

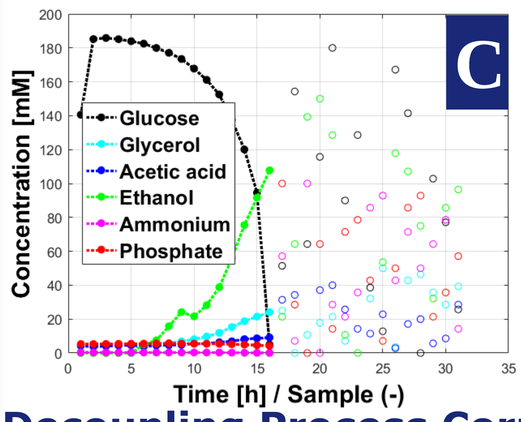
Industry is increasingly focusing on the development of more efficient and less time-consuming methods to **monitor and control** their **fermentation processes** at optimal conditions. Here, **Infrared (IR) Spectroscopy** is among the most promising techniques for on-line or at-line applications. Fitted to the fermenter by fiber optical probes, IR spectroscopy **coupled with mathematical models** relates the spectra to various components of interest. A measurement is completed **within a minute** and a **variety of nutrients** and metabolites can be detected in a single spectrum. Within this approach, **PLS-models** have been developed to **predict** the concentration of **Glucose, Ethanol, Glycerol, Acetic acid, Ammonium and Phosphate** in a yeast fermentation process.

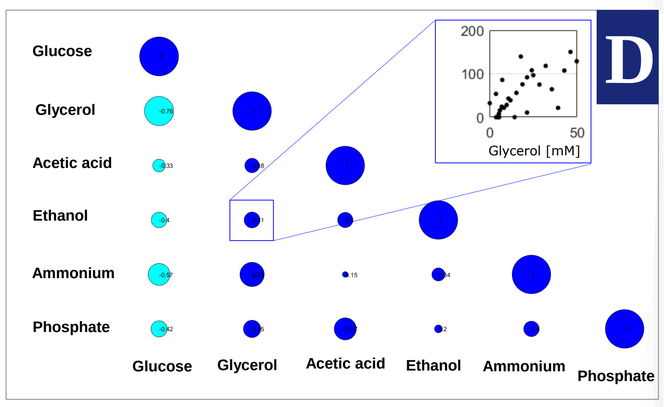
The method has been developed on a lab-scale fermentation setup, using **YPD medium** as a complex nutrient source, adjusting the operating conditions to mimic industrial operation. Our calibration models are built on both several **yeast batch-fermentations**, representing relevant different process conditions, and **synthetic samples** serving the need of decoupling the natural correlation dynamics of the target species. Additionally, IR spectra were collected with a classical **FTIR** instrument and the novel patented upconversion technology **NLIR.** The NLIR technology is considered for at-line application and models built on both spectral datasets were **compared regarding their performance**.

Preparation





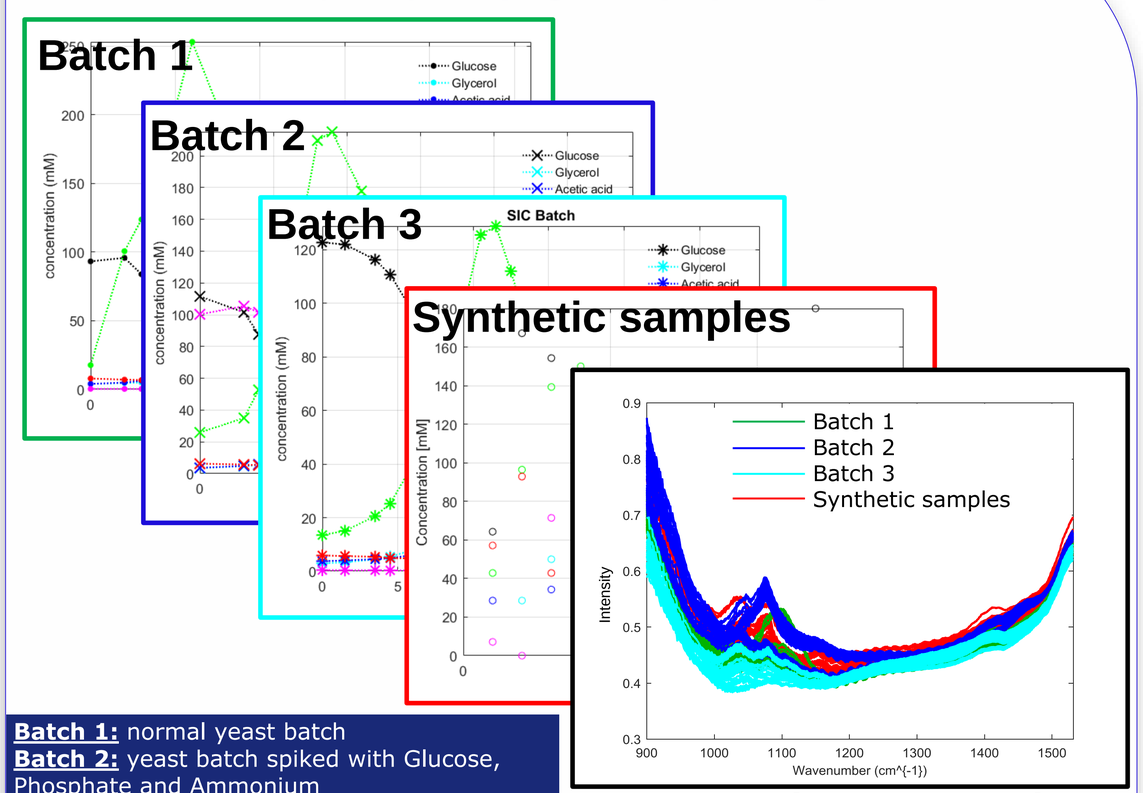




Decoupling Process Correlations

Batch cultivations generally show a quite high level of correlation between the different species consumed and produced (**A, B**). A powerful way to decouple the correlations is by means of unique, synthetic samples added to the calibration set (**C, D**).

Data Collection



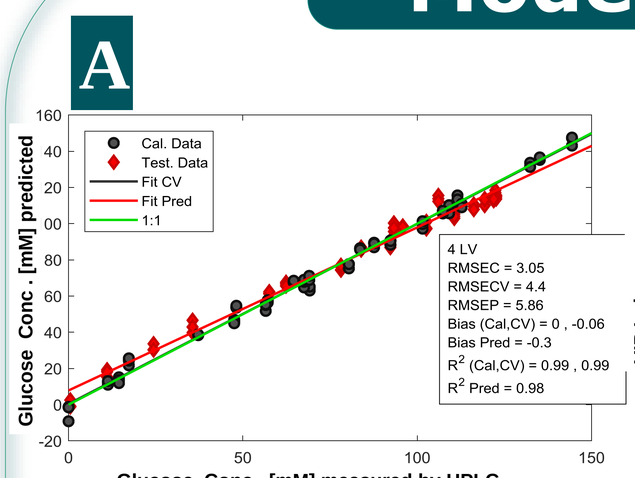
Batch 1: normal yeast batch
Batch 2: yeast batch spiked with Glucose, Phosphate and Ammonium
Batch 3: normal batch, insulin producing yeast

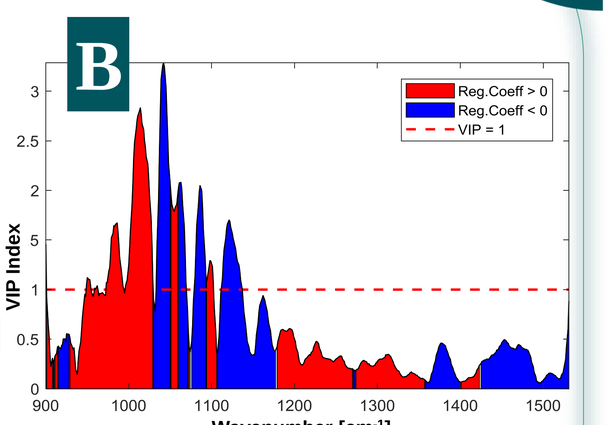
IR Spectra (NLIR sensor)

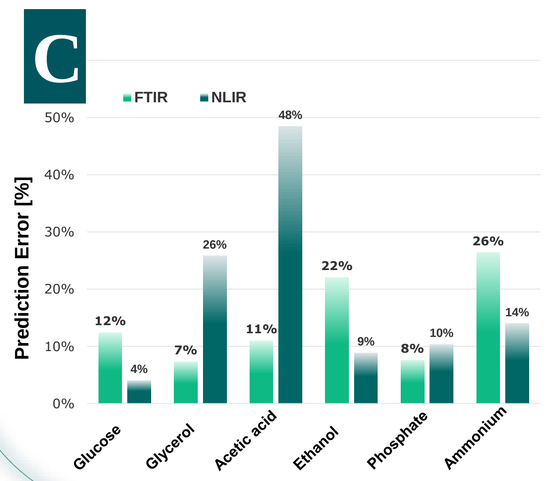
Synthetic samples:
15 samples containing the 6 analytes of interest (Glucose, Glycerol, Acetic acid, Ethanol, Ammonium, Phosphate) in artificial proportions chosen to disturb the natural correlations.

The different data sets have been divided into data sets used for calibration (Batch 2 + synthetic samples) and validation of the models (Batch 1 and 3).

PLS Modelling





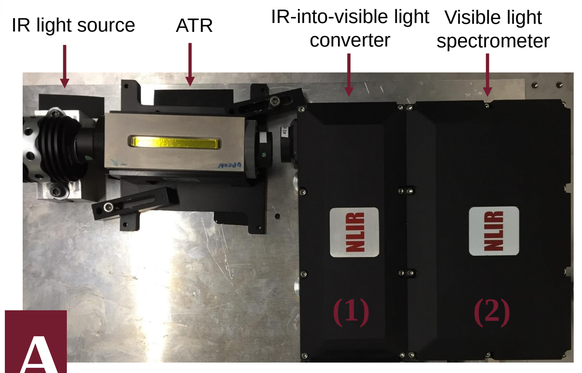


A: Exemplarily shown is the Partial-Least-Square (PLS) Model for Glucose based on the NLIR spectra. Calibration (Cal.) data were Batch 2 and the synthetic samples. Validation (Test) data were Batch 1 and Batch 3.

B: Model Evaluation: VIP index and regression coefficient for the different Variables (Wavenumbers): a VIP index >1 indicates a high relevance for the model; Positive correlation coefficients of the variables indicate a positive correlation between the respective variables and the Glucose concentration.

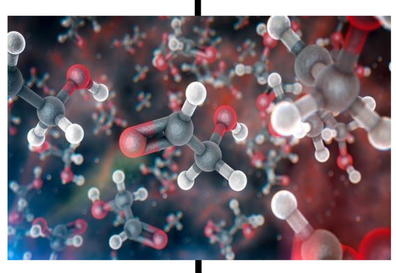
C: Comparison of the prediction error of the models built on FTIR and NLIR data.
Spectra of all data sets were collected with both, a common lab FTIR (Fourier Transform IR) instrument and a NLIR (Non Linear IR) instrument considered for online application due to increased detectivity.

NLIR Technology



- **higher sensitivity,**
- **10 x faster than FTIR**

Mid-infrared light **B**



Non-linear upconversion

$$\frac{1}{\lambda_{up}} = \frac{1}{\lambda_{MIR}} + \frac{1}{\lambda_{1064}}$$

CCD/CMOS camera

A: Setup for mid-infrared spectroscopy. A hot body (IR light source, 900 °C) emits IR light that passes through ATR and then into the NLIR sensor unit (1+2). The NLIR (1) box mixes the IR light with a high-power laser field (100 W) at 1064 nm and thereby converts the IR light to near-visible wavelengths. The NLIR (2) box is a near-visible grating spectrometer that measures the spectral content of the upconverted light.

B: Illustration of the NLIR technology. IR light interacts with molecules, is upconverted with spectral information conserved due to energy conservation, and finally detected using a standard Silicon-based detector (CCD/CMOS).
This is in contrast to a conventional FTIR spectrometer that measures the IR light directly with a much lower detectivity.

BIOPRO

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